Summary

Agriculture is essential to feed our ever increasing human population and depends largely on healthy and fertile soil. Aboveground production of crops, or plants in general, is inextricably linked to a complex web of an astonishing number of organisms, chemical reactions, processes and interactions; the soil ecosystem. Especially the decomposition of dead organic matter is one of the most important soil processes which returns essential nutrients to the soil ecosystem, ready to be taken up by plants. Decomposition is, amongst other things, dependent on microbes and soil invertebrates, including earthworms, isopods and many other species, each performing a particular function. The identity of species and functional diversity within the soil community, rather than the number and biodiversity of species, drive soil ecosystem processes. The loss of a single species that represents an essential soil function may, therefore, have serious consequences for soil ecosystem functioning, quality and plant production.

Soil ecosystems can be challenged by many factors, including the implementation of novel crops on an agricultural field. Novel crops can, for instance, arise via genetic modification (GM). As with any new technology, GM raised many concerns about potential undesired ecological impacts, such as effects on essential soil invertebrates. This thesis therefore, focused on how novel (GM) crops can affect the soil ecosystem and how potential risks can be assessed.

As a model for novel GM crops, *Brassica oleracea* was chosen. Many of our daily vegetables belong to this species, such as broccoli, cabbage and Brussels sprouts. *Brassica* species are well–known for to their anti–herbivore defence strategy involving the production of glucosinolates (GSL). Tissue damage of these crops, due to for instance chewing by herbivores, converts the GSL into several toxic compounds, of which the isothiocyanates (ITC) are the most well–known. ITCs have shown indistinct toxicity towards insect herbivores and could thus potentially also have detrimental effects on beneficial soil invertebrates. Recent studies discovered that ITCs are anti–oxidants and, for instance, show anti–carcinogenic activity in humans. Furthermore, plants containing GSL are used as an alternative to synthetic pesticides for pest management on agricultural fields, making use of the natural toxicity of these plants. This practise is better known as biofumigation. These socio–economic benefits have raised the demand for crop with elevated GSL levels, which can be obtained via GM. However, before such novel GM crops are allowed in the field, potential risks towards the environment have to be investigated.

The aim of my PhD was therefore to develop a practical tool, based on scientific studies, which should aid governmental decision makers in the assessment of potential risks of novel (GM) crops towards soil ecosystems. To that end, a decision matrix was developed, representing a tiered approach to investigate potential adverse effects of GSL and their hydrolysis products at different organizational levels (from molecular to community). This approach combined the research fields of toxicology, genomics and community ecology. Five key questions formed the backbone of this thesis: i) What are the effects of ITC and GSL–containing plant material on survival and reproduction of soil invertebrates? ii) What is the underlying mechanism of the toxic effects on a gene expression level? iii) What is the effect of GM compared to non–GM plant material on soil invertebrates? iv) What are the effects of GSL containing plant material on soil invertebrate communities? v) How do results found in laboratory studies compare to field baseline data?

To answer the first question, effects of 2–phenylethyl GSL hydrolysis products were investigated for four soil invertebrates; the springtails *Folsomia candida* (fungivore), *Protaphorura fimata* (facultative herbivore), the earthworm *Eisenia andrei* and the isopod *Porcellio scaber*. Using internationally standardized guidelines, experiments with 2–phenylethyl ITC demonstrated that these natural toxins can have serious impacts on survival and reproduction of soil invertebrates. These effects were within environmentally relevant concentrations found in agricultural soils. Concentrations much lower than reported in soils after biofumigation, for instance, easily reduced 50% of the reproduction and survival of the invertebrate species investigated. This illustrates the toxic character of these compounds on soil invertebrates. Results, furthermore, showed that springtail species were the most sensitive to ITC compared to earthworms and isopods. Moreover these natural toxins are not target specific, affecting both herbivore (pest species) and non–herbivore (non–target) species to a similar degree.

Besides tests with specific ITC, experiments were conducted using material from plants which varied naturally in GSL concentration and composition. In this setting, soil invertebrates were exposed to a wide range of ITCs simultaneously via fragmented plant material mixed through soil (biofumigation). These experiments confirmed that increased total concentration of GSL (and thus ITC), decreased survival and reproduction of soil invertebrates, although earthworm survival was not affected. Moreover, the soil microbial community was investigated over time following exposure. A peak effect on microbial communities was found after one week, with

subsequent restoration to original communities taking place. Overall, the microbial community therefore seems quite resilient to GSL stress conditions.

In order to study the underlying modes of action of 2–phenylethyl ITC, gene expression profiles of the springtail *F. candida* and earthworm *E. andrei* were investigated. Comparing such profiles obtained under control and stressed conditions, illustrate changes in the physiological status of an organism at a particular time. For springtails, the importance of changes in fatty acid metabolism was revealed, reflecting the lipophilic character of the compound. Furthermore, a direct link to reproductive impairment was found among the affected genes of springtails, while this was not apparent in earthworms. In worms, ITC mainly affected chitin production and induced several general and specific oxidative stress related responses. Gene expression profiles found in these studies yield valuable leads for early warning signals of ITC toxic stress in soil invertebrates.

High GSL levels in *B. oleracea* plants occur naturally, but can be further enhanced via genetic modification (GM). To understand if GM per se could affect soil invertebrates, species were exposed to GM *B. oleracea* plant material of several GM plants developed during the project. Effects of GM plant material were tested via biofumigation (mixing through soil) and directly compared to non–GM material. Most of these GM plants did not show any additional negative effects on soil invertebrates compared to naturally occurring *B. oleracea* plants. Only one GM plant was more toxic due to the increase in concentration of one specific GSL, whereas total GSL concentration was the same as non–GM natural varieties. These results show that it is not GM per se, but GSL specific concentrations that could potentially form a risk towards the environment.

In order to better reflect the complexity of soil ecosystems, experiments with *B. oleracea* plant material were conducted using various ensembles of soil invertebrates in microcosms. Using either plant material with high or low GSL concentration, effects on invertebrate survival and soil processes (CO_2 production) were measured. This set up would reveal if particular species and soil processes would perform better or worse in the presence of other species and under stress of GSL. Exposure to high GSL plant material, reduced springtails survival, but at the same time, springtail survival was positively affected by the presence of earthworms. Soil processes remained largely unaffected mainly because earthworms performed well under high GSL conditions. These results confirm the important role of earthworms within the soil ecosystem. Their well–being is therefore of utmost importance when considering the introduction of new crops.

This thesis ends with a general discussion to put the obtained results into perspective of what could potentially be found in the field. This is because ecological risk assessment (ERA) is a combination of hazard impact (toxic potential) and the likelihood of exposure to the hazard. Thus comparisons of laboratory obtained data to field conditions including natural variations found in the field, so–called baseline data, were made. In the case of GSL (in general), exposure can occur via root systems of growing plants, decomposition of dead plant material, but also via intentional mixing of material into the soil (biofumigation). Field data on GSL are currently very limited, hence the assessment of potential risks is hard to predict and deserves more attention. Nonetheless, exposure via roots and decomposition of dead plant material seem harmless. Biofumigation practises however, have shown to reach concentrations that are toxic to the soil invertebrates investigated within this thesis. Especially as techniques to increase the efficiency of GSL hydrolysis in soil, and thus toxicity, are improving rapidly, care should be taken in order to maintain a healthy soil ecosystem.

An important issue to consider is the stability of GSL and ITC in soil. It is generally known that both compounds disappear rapidly from the soil via evaporation or degradation into harmless compounds. This was also studied within this thesis, showing that concentrations of 2–phenylethyl ITC were halved within, on average, 18 hours. This suggests that soil ecosystem communities are only exposed for a very short time to toxic conditions, created by GSL containing plant material, and may be able to recover quickly after GSL release.

Field experimental data on GM *B. oleracea* plants is, to my knowledge, currently non–existent, primarily due to the very strict legislation surrounding GM crops. Such data is essential to study, for instance, long–term effects and indirect impacts of GM crops which may arise from changes in agricultural managing practises compared to conventional crops. What should be noted is that soil ecosystems could also actually benefit from GM crops due to changes in agricultural management. For instance, with some GM crops the use of synthetic pesticides is decreased, providing more advantageous conditions for beneficial soil invertebrates and thus a healthier soil ecosystem.

In conclusion, the decision matrix developed within this thesis offers a comprehensive overview of potential adverse effects of novel (GM) crops on multiple organizational scales ranging from molecular to soil process effects. The decision matrix can thus be a useful tool for risk assessment. Moreover, its applicability can be extended to other plant traits and environmental conditions that are potentially hazardous to the soil ecosystem.